compartmentalization, DNA methylation state, and chromatin status can all conceivably contribute to dynamic changes in the equilibrium of a general TF binding to either classical or tissue-specific sites. Since, as the authors also suggest, CRX regulates MEF2D activity in an additional, DNA-binding independent mode, dissecting what specific elements and/or additional factors contribute to the functional interactions of CRX with MEF2D could help to elucidate this whole new dimension of tissue-specific gene regulation.

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Unraveling a Locomotor Network, Many Neurons at a Time

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In this issue of *Neuron*, Bruno et al. (2015) use large-scale recordings in *Aplysia*, and apply novel dimensionality-reduction techniques to define dynamical building blocks involved in locomotor behavior. These techniques open new avenues to the study of neuronal networks.

One key goal of neuroscientists is to understand how neural circuits produce behavior. While circuit function has been studied in a multitude of species, including humans, it is arguably studies of invertebrates that have yielded the greatest insights into underlying circuit mechanisms.

Through studies of the mollusc, *Tritonia*, in the 1970s and 1980s, Peter Getting established a sequential 8-step approach to circuits producing rhythmic movements (Getting, 1986): (a) describe the behavior; (b) characterize the motor pattern; (c) identify the neurons involved; (d) localize the key neurons involved; (e) map the synaptic connectivity; (f) characterize the cellular properties; (g) manipulate the network; and (h) reconstruct the network. In the ensuing 30 years, great strides have been made in invertebrates and vertebrates alike in at least the first three steps and to varying degrees in the others (Brownstone and Wilson, 2008).

Although this step-wise approach is quite logical, there are several meta-problems with it. For one, individual neurons may be involved in more than one motor behavior, meaning that there are not specific circuits dedicated to each motor program (Getting, 1989; Wu et al., 1994). In addition, in all but the simplest nervous systems, many dozens to hundreds to thousands of neurons may be involved in producing the activity, presenting a key stumbling block in the capacity to simultaneously record large numbers of neurons. And if this could be accomplished, how is the large volume of data then to be analyzed? In other words, a major stumbling block in understanding the CNS is its high dimensionality. In order to understand these networks, it is necessary to parse these large datasets using methods aimed at reducing their dimensionality (Cunningham and Yu, 2014; O'Leary and Marder, 2014; Vogelstein et al., 2014). So while the linear approach proposed by Getting (1989) is particularly well-suited for conventional analysis, it implies a reductionism that does not necessarily pair with the multidimensionality of the CNS.

In this issue of *Neuron*, Bruno et al. (2015) use new techniques in a traditional preparation to ask how large numbers of neurons assemble to produce a behavior. They studied the escape motor program in *Aplysia*. By mimicking a noxious stimulus applied to the tail, they induced rolling waves of dorsal and ventral activity along the antero-posterior axis of the animal. This locomotor behavior is produced by the pedal ganglion, which contains ~1,600 neurons, including pattern generators, modulator neurons, and motoneurons. Bruno et al. (2015) used an approach in which they combined large-scale recordings with high temporal and spatial resolution to simultaneously record dozens of neurons. They then reduced the

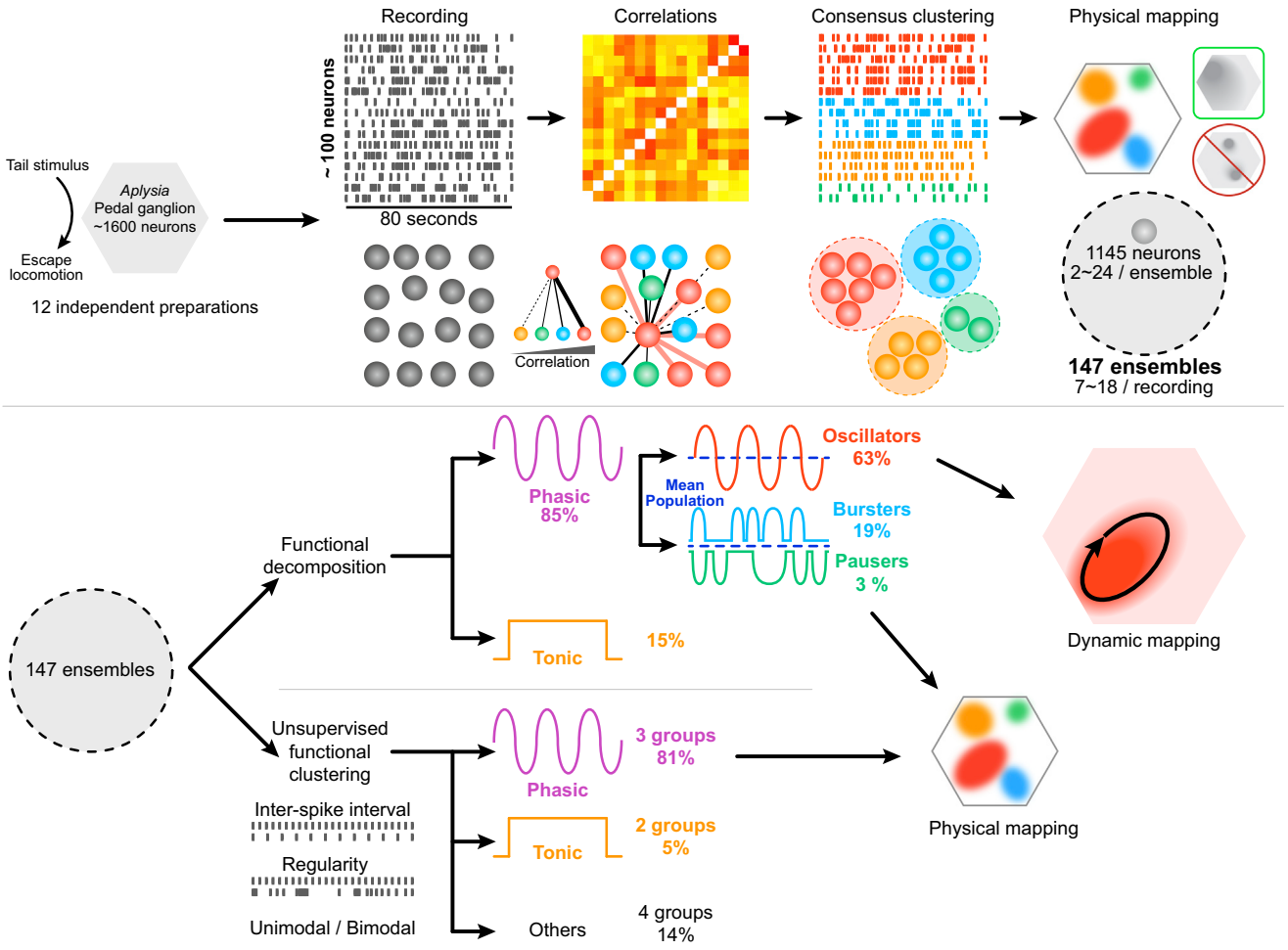

Figure 1. Dimensionality Reduction of Locomotor Network: Neurons that Tick Together Stick Together

dimensionality of the data to provide insight into circuit operation.

Their first step was to decompose the motor program (see Figure 1), akin to Getting's second step. Between 57 and 125 neurons were simultaneously recorded using a voltage-sensitive dye. The authors first processed the recordings using independent component analysis (ICA) (Hill et al., 2010) to extract neuronal spikes from the voltage imaging data. Initial principal component (PC) analysis, however, revealed high variability in the number of PCs needed to account for 95% of the variance, suggesting that different dynamics were captured in each recording. They then grouped neurons based on their firing properties into correlated ensembles based on consensus clustering designed to maximize the correlation within a group and minimize the correlation between groups. Next, they asked whether they could identify in the recordings ensembles that could then be applied across recordings. These resulting ensembles formed the basis for decomposing the dynamical systems within the motor program.

How did they detect ensembles? Using an unsupervised analysis of the activity patterns of each neuron, they grouped neurons with similar activity patterns into clusters: they developed a method of "consensus community detection." Because of the outrageous number of possible groups ($\sim 4.75 \times 10^{115}$ for 100 neurons), a clustering algorithm samples a smaller number of randomized iterations to reach a convergent configuration. The authors designed a parameter-free consensus community detection algorithm that identifies consistent optimal solutions across trials. This method enabled the identification of ensembles that were coherent across multiple trials.

In each recording, there were between 7 and 18 ensembles (147 total), each comprising between 2 and 24 neurons. Remarkably, when they mapped these ensembles onto the physical structure of the pedal ganglion, they found that the majority of the ensembles formed contiguous physical clusters. That is, neurons that tick together, stick together.

They then classified the ensembles according to their activity, with 85% being phasically active. Among them, 63% were oscillatory with periodic phases of activity above and below the mean activity of the entire population. An additional 19% were classified as "bursters"—neurons with intermittent bursting episodes exclusively above the mean activity. 3% had only recurrent drops of activity below the population mean and were therefore

classified as "pausers," but these were too infrequent for further analysis. The remaining 15% of neurons were tonically active during the task performance. With this classification, it was possible to project each of the classes onto a low-dimensional space (first two PCs) in order to visualize population dynamics.

The oscillator class was shown to have a constant rotation in this space, whereas the burster class activity evolved as the behavior progressed. Thus, there were at least three dynamic systems involved (including the non-oscillators). Importantly, the constant rotation of the oscillator class was preserved despite variations in the motor program between executions. These oscillation classes also mapped onto discrete regions of the physical space, and in fact, the rotation of the oscillator class in PC space translated to rotation in physical space demonstrating low-dimensional rotational dynamics. Interestingly, mapping the dynamic firing activity of identified oscillators revealed an ellipsoid structure, demonstrating that this escape locomotion is not a simple alternation of two phases, but instead a continuum with slower dorsal extension and faster ventral flexion. This can be seen in the video, which shows an orbital ellipsoid that accelerates during ventral flexion and decelerates during dorsal extension. The authors mused that this result might mean that these neurons were the rhythm-generating kernel of the network. But while this behavior might be necessary for a rhythm-generating kernel, it is not sufficient to define one.

The authors didn't stop here. As they had applied constraints in defining the oscillation patterns, which enabled isolation of the dynamical systems within a recording, they then used a different approach to ask whether their constraints influenced the results. They thus repeated the decomposition using a different unsupervised algorithm analyzing the spike-train structure: firing rate and regularity. To do this, they fit six models (four unimodal or tonic, two bimodal or phasic) to each distribution then computed the best fit. Each ensemble was then analyzed in 12-dimensional space (six models by rate by regularity) and the distance between each pair used for unsupervised analysis of clustering. This defined groups of ensembles based on similarity of spike trains. Across all recordings, they found nine ensemble groups (2–6 groups per recording); that is, the 1,145 recorded neurons were first reduced to 147 ensembles, and then to nine fundamental dynamical systems. Again, this unsupervised analysis recapitulated the previous result showing that the vast majority of ensembles gathered into an oscillatory group in the caudolateral area of the pedal ganglion. Thus, they showed that the ensembles were independent of experimenter constraints and furthermore that this technique allowed for combination of data from all recordings. This combination provided an increase in statistical power and the resultant identification of ensembles that may only appear in a few recordings.

Thus, by using two different and independent approaches to clustering neurons into ensembles, Bruno et al. (2015) have determined that there are a discrete number of physically aggregated ensembles—"dynamical building blocks"—that comprise the motor program. They demonstrate a rhythmic network and locate this in the caudolateral quadrant of the ganglion. While rhythm-generating networks must have cyclical attractors (a single pacemaker neuron is a cyclical attractor), the authors provide evidence that in *Aplysia*, the rhythm is generated through a cyclical attractor network. To further understand this, it would be important to know what happens during disruptions of the rhythm. That is, if the rhythm were perturbed, would the cyclical attractor reset and move back to its rhythm?

To further test these analysis methods, these techniques could readily be scaled to a number of different systems to gain further understanding of neural circuit function. It is commendable that the procedures are not only well documented in the supplementary material, but the authors have made their code available.

This manuscript demonstrates the possibilities of strong mathematical analysis of multi-neuron recordings. This does not negate the importance of studying circuits one or a few neurons at a time; to understand mechanisms underlying circuit function, we must understand biophysical properties of neurons and synapses (Harris-Warrick, 2002; Kristan and Katz, 2006; Russell and Hartline, 1978). The complementary approach presented here, however, could guide single-cell recordings toward particular regions and specific neurons in order to determine the neuronal properties and connectivity that underlie behavior. In other words, this study provides an approach to Getting's steps of understanding the mechanisms of neural circuit function. That is, while this manuscript does not speak to the cellular mechanism of network function, it beautifully defines its phenomenology and provides a new analytical framework with which to decode complex behaviors.

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